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THE PROBLEM OF IMMUNISATION OF DOMESTIC ANIMALS TO HELMINTHOSES

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Studies on the immunity to helminthoses were initiated in this country in the early 30 and resulted in a number of works carried out by K. I. Skryabin and his collaborators. The first report on the subject was published in 1935 by R. S. Schults and N. P. Shikhobalova and was followed by many review articles (1). There are also many papers dealing with this problem in other countries (2). Though the point in question is far from being fully investigated, one can however affirm that the immunity to helminthoses does exist and develop in accordance with general physiological laws. Helminths exert an influence on the host's organism that responds to uncommon irritations by an enhanced reactivity to produce protective adaptation resulting in stability and limiting pathogenecity and viability of helminths. Numerous investigations have proved the possibility of an active immunisation of animals to a number of helminthoses with antigens prepared from helminths. O. Wagner (1933) studied the active immunisation of white mice to ascaridiosis and Oliver-González (1943) dealt with rabbits. L. K. Melcher and D. N. Campbell (1942) immunised laboratory animals to trichinolisis with polysaccharide antigen prepared from Trichinella larvae and J. F. Sprent and H. H. Chen (1949, 1951) treated them with different chemical fractions isolated from tissues of Ascaris.

(1) Such as those by R. S. Schults and N. P. Shikhobalova (1937, 1940); G. S Markov (1938, 1939, 1941, 1948); N. P. Shikhobalova and E. S. Leikina (1949); N. P. Shikhobalova (1950); R. S. Schults and E. A. Davtyan (1948, 1949), etc.

(2) For example those by J. H. Sandground (1928, 1929); J. H. Ackert (1932-1946) E. L. Taylor (1934), J. T. Culbertson (1945); A. C. Chandler (1932-1948), etc.

Unfortunately these experiments as well as many others, though devoted to the study of immunity and immunisation to helminths, were carried out on laboratory animals known to be non-specific hosts. As to the active immunisation of domestic animals to helminthoses R. E. Rebrassier and B. F. Mc-Crory (1931) investigated the immunisation of chickens to ascaridiosis with an extract from dried Ascaridia. L. K. Eisenbrant and J. E. Aekert (1940) used to the same end a sterile extract from Ascaridia galli I). In this country a great deal of work has been recently done concerning the immunisation of domestic animals to a variety of helminthoses. V. I. Pukhov and P. A. Velitchkin (1936) studied the immunisation of sheep to monieziosis and met with success when they had made use of extracts from Moniezia. V. I. Pukhov, E. E. Krivoshta and Reshetnyak (1950) caried out immunisation of sheep to "husk" (dictyocaulosis) with extracts from Dictyocaulus.

To immunise sheep against lungworms E. A. Davtyan and R. S. Schults (1948. 1956) injected live infective or invasive larvae of Dictyocaulus both intravenously and intramuscularly. The results obtained led these authors to suggest this retratment as a method of active immunisation, subject to further study. E. S. Leikina (1944, 1946, 1948) dealt with active immunisation to helminthoses by applying the reaction of precipitation to determine the presence of antibodies produced in pigs at different stages of ascaridiosis.

K. Matov (1955) studied the active immunisation of dogs to echinoccosis.

As a result of the reported investigations one can conclude that the immunity to helminthoses is characterised by *a*) complete immunity to the corresponding helminthoses in a part of immunised animals; *b*) limited infectivity (the immunised animals harbouring fewer parasites); *c*) retarded development and lowered reproducibility of parasites; *d*) reduced viability of eggs and larvae of helminths ejected by the immunised animals; *e*) reduced pathogenecity of helminths.

To test the immunisation one used various antigens contained in fresh and preserved helminths, their metabolites, antigen complexes or separate chemical fractions (polysaccharides or proteins) of parasites, antigens prepared from the larvae forms of helminths and those prepared from different tissues of parasites. Some workers used as antigens live helminths. mainly their infective eggs or larvae. There is up to now no unambiguous opinion on the advantages of particular antigens. The immunisation of animals with living material (infective eggs and larvae) gives in respect to particular helminthoses better

(1) These of course are only two of a number of works on active immunisation of specific hosts.

results than that with antigens prepared from dead helminths, but immunisation with living material is dangerous in that it creates the danger of helminths developing to a definite, even to the imago stage. When immunising with living material to avoid infection of animals one uses weakened strains, repeated injections of infective larvae, etc.

The most thoroughly studied fractions are proteins and polysaccharides.

At its present stage helminthology has not enough knowlege of the physiology and biochemistry of helminths. When there is enough additional information, it will be possible to produce more active antigens. As to the methods, there are up to now satisfactory ones concerning the technique of preparing antigens, determining their immunising properties, their exact dosage, etc. It is not unfrequent that workers using the same procedure of preparing an antigen get after immunisation opposite results owing to the lack of conventional techniques. Of many methods to prepare antigens the best one seems to be that of producing polysaccharides from helminths due to D. N. Campbell (1936, 1937). To obtain active antigens from helminths S. N. Babadjanov (USSR) proposed in 1947 a modified Boivin method (1933) used to produce bacterial antigens. In 1955 O. I. Polyakova suggested the following method to prepare antigens. Fresh helminths are washed in distilled water, ground and dried in high vacuum at —35 to —70° C. (lyophylisation). To remove free lipoids the dried substance is treated in a Sockslet apparatus with absolute alcohol and then with ether - The substance deprived of fat is ground in a mortar. The dry helminths powder is dispersed in water in ratio 1 : 10 (by weight) with an equal volume of 40 % glucose solution being added. The mixture is stored in a thermostat a 37° C. for 48 hours and then centrifuged. the precipitate being re-extracted with 20 % glucose solution and once more centrifuged. The solutions are dialysed from glucose, the dialysate being then condensed in vacuum to a small volume and the antigen precipitated with a five-fold volume of absolute alcohol. After standing in the cold the precipitate is isolated by centrifuging, washed with absolute alcohol and dried in a vacuum desiccator.

O. I. Polyakova (1958) has carried out at the Institute of Helminthology a comparative study of antigens prepared from D. filaria by three methods (due to Boivin, Melcher and Polyakova respectively) and has also determined the chemical composition of antigens from Ascaris, Fasciola, Dicrocoelium, Thysaniezia and Trichinella prepared following Boivin (see the Table below).

HELMINTHS	Method of preparation of antigens	Nitrogen (per cent)	Reducing substances calculated for glucose		Glucosamine (per cent)
			before hydrolysis (per cent)	after hydrolysis (per cent)	
Dictyocaulus filiaria.	Boivin	1,8	3,2	58,2	2,3
»	Melcher	5,1	3,4	62,9	0
»	Polyakova	2,9	1,7	55,1	2,4
Ascaris lumbricoides.	Boivin	2,7	1,9	57,4	1,8
»	Melcher	5,2	2,2	72,6	0
Fasciola hepatica ...	Boivin	1,3	1,9	75,3	2,2
Dicrocoelium lanceatum	Boivin	0	3,05	82,3	0
Thysaniezia ovilla ...	Boivin	1,4	0,8	91,6	0,61
Trichinella spiralis (larvae)	Boivin	1,9	0,6	88,9	3,1

Data listed above show that all antigens are characterised by a sharp increase in reducing substances due to acid hydrolysis. This points to a high percentage of the polysaccharide component present in the complex. The presence of glucosamine shows this polysaccharide not to be glycogen but one specific polysaccharides. An exception to the rule is the antigen from Dicrocoelium, as it has been found to contain no glucosamine nor any nitrogen whatever. The preparation produced following Melcher are essentially polysccharides not forming a complex with proteins or peptides. These preparations are however contaminated with nitrogen containing admixtures, being unsatisfactorily purified.

Polyakova was led to conclude that the antigens isolated from helminths following Boivin and the author are polysaccharide-peptide complexes in which the carbohydrate complex predominates. The latter is a specific polysaccharide consisting of glucose and glucosamine and is therefore different from a common reserve carbohydrate of animals that is glycogen. To obtain antigen from helminths. V. S. Ershov worked out (1950) a modification of Alexandrov and Hefen method used by them to prepare antigens from bacteriae. Following V. S. Ershov the method of obtaining antigens is to effect the maximum fermentative degradation of cell structure. the digestion of bulky helminths proteins with pancreatin and to treat the digested mixture with alcohol to obtain a chemically stable complex with the antigen, essentially a polysaccharide.

Fresh helminths from animals slaughtered at a meat factory were washed with water and weighed. They were then finely minced or ground in a china mortar and immersed in distilled water or physiological solution in the 1 to 3 ratio (by weight). To the resulting mixture were added 7 to 10 g. per 1 l. of

active pancreatin depending on its activity (not less than 25 active units (Metta tests) being allowable).

To the mixture is added 10 to 15 g. sodium bicarbonate and 10 ml. chloroform per 1 l. Helminth tissues were dissolved in alkaline medium at pH 7.4 to 8.2 in a thermostat at 38 to 40° C. The digestion period lasted up to 3 days for Fasciola, 12 to 14 days por Ascaris and 7 to 9 days for Ascaridia.

Every 1-2 hours the bottled mixture was shaken without offsetting the cork. During hydrolysis the pH was continually checked for its drop to acid reaction necessitated the addition of sodium bicarbonate. By the end of the process the helminths had been completely dissolved and the emulsion became clear, only the eggs remaining in the precipitate. The liquid was then syphoned off to leave the precipitate, centrifuged at 2,000 r. p. m. for 3 to 5 minutes and placed in a five-fold volume of 96° alcohol. In few minutes there appeared a flaky precipitate that took from 15 to 20 hours to condense. In 24 hours the alcohol was syphoned off and the precipitate, with antigen was poured into Petri dishes and dried either in a thermostat or in vacuum.

Workers of the Institute of Helminthology (V. S. Ershov, Z. A. Arzimowich, V. I. Predikhina, U. J. Dol'nikov, D. N. Dubovoij, E. I. Malakhova, and M. I. Naumitcheva) have been applying from 1950 this method. They prepared antigens from *Ascaridia, Toxocara, Ascaris suum, Fasciola* and *Moniezia* expansa, studied their chemical structure, proved their antigenic and immunological properties on laboratory animals and have finally used them for active immunisation of domestic animals.

Antigens prepared following the above method are a polysaccharide-albumin complex involving up to 60 % of the former and 30-40 % of the latter component. To settle the question whether these antigens were in fact antigens one of above workers. M. I. Naumitcheva, has proved (1954) by applying to laboratory animals anaphylaxis and allergy method that substances prepared from Ascaridia galli, Toxocara and Toxocaris of dogs, Ascaris suum, Fasciola and Moniezia of sheep are indeed antigens. They are highly specific and display the properties of antigens. Sensibilisation of guinea pigs with corresponding antigens results, as a rule, in a typical anaphylaxis reaction. In the experiments with antigen cross checking no anaphylaxis was observed.

The respective antigens lead in laboratory animals and specific hosts to the appearance of precipitators and give a positive precipitation test with homologous antibodies and antibodies from animal blood serum previosly infected with corresponding helminths. The maximum titres of precipitators were noted 7 to 14 days after the last injection of antigen. Hystological data (N. P. Tsvetayeva of the Institute of Helminhology) have subsantiated the enhanced

reactivity of chicken as compared to that of control nonimmunised chicks that had recovered from the first intensive infection.

The toxicity of antigens was studied on white mice (live weight up to 15 g.), rabbits, and specific hosts. As a lethal dose was considered one that led in 24-36 hours after subcutaneous injection to the death of all mice, there being a definite patho-anatomic specificity. Before being administered each antigen series was checked as to its toxicity on white mice. The antigens from helminths did not prove to be comparatively toxic. It was found that for white mice the lethal dose amounted to 75 mg. of antigens prepared from Ascaris, 300 mg. from Ascaridia, 200 mg. from Moniezia, and 200 mg. from Fasciola. The antigen dose both optimal and minimal to be used for immunisation was previously titrated on laboratory and then specific animals.

The immuno-biological poperties of antigens were first tested on laboratory animals, their general and local action being studied on respective hosts.

The minimum of antigen injections to give the best immunity effect was investigated on corresponding host-animals by injecting them two to three times with a lethal dose of infective eggs or larvae (adolescartia of Fasciola, cysticercoida of Monezia).

The determination of the duration of immunity to respective helminths was carried out by infecting the immunised animals at different intervals.

a) *Immunisation of young pigs to ascaridosis.*

Tests undertaken by V. S. Ershov, D. N. Dubovoy et al. on 173 pigs two-three months of age, gave the following results. The effective immunisation dosage proved to be the first antigen injection of 0.3 g. and the second - of 0.5 g., administered subcutaneously with a 12 days rest period, these dosages resulting in 78-80 % immunity to ascaridosis. The other immunised pigs experienced lowering in infectivity. When being well fed after they had been given antigen and the introduction of infective eggs of Ascaris, the immunised pigs showed no abnormal characterics but for the drop in the live weight. In a few minutes after the administration of antigen some pigs experienced reddening of the skin on the head, neck, and ears, acquired intensified breathing, vomited, showed signs of general agitation. Such a reaction lasted for 30-40 minutes. The antigen being injected for the second time. this reaction was absent or was much weaker, lasting only for 2-3 minutes. The controls on normal diet, experienced in 5-7 days after being infected signs of the early stage of ascaridosis and lost considerably in their live weight. After being treated with antigen young pigs retain immunity to ascaridosis for 4-6 months. This immunisation can be effected however only if there is enough vitamin A in the diet. Both immunised and control pigs not receiving A-vitamin displayed

on the 5th day after infection ever growing uneasiness and some of them died. Without vitamin A in the diet the immunisation of young pigs to ascaridosis is not effective.

b) *Immunisation of chickens to ascaridosis.*

Tests involving 300 chickens carried out by U. J. Dol'nikov led to following results.

Effective antigen doses for immunising 30-50 days old chicks proved to be those of 100-200 g. injected subcutneously in portions together with calcium phosphate with 7 to 14 days rest period. The above dosage accounts for complete immunity to ascaridosis of 28 % of chickens. Other immunised chickens showed lowering in infectivity as well as retarded growth and sexual development of Ascaridia. All controls had been 100 % infected, the invasion being very intensive. Immunised and superinfested chickens had a very high immunity against infection with intensive doses of Ascaridia eggs. Hystological evidence has shown the immunised chickens to posess a higher reactivity and better weight characteristics than the controls, immunisation not affecting the gain in weight. After being artifically infected the immunised chickens experienced mostly a higher increase in weight than the nonimmunised controls.

The immunisation of chickens with antigen provides immunity to ascaridosis only in the case there is enough of the A and B vitamin complex and vitamin D in the diet, immunisation not being otherwise effective.

c) *Immunisation of lambs to monieziosis.*

Tests carried out by V. I. Bredikhina gave the following results.

Effective dosages to immunise lambs proved to be as follows: the first - 200 mg., the second - 300 mg. and the third one - 500 mg., all of them injected intramuscularly with 7 to 10 days rest periods. These doses lead to a complete immunity in 60 % of the lambs to moniezosis and retard the development of Moniezia in the remaining 40 %. The immunisation with antigen holds good for 1-5 months.

d) *Immunisation of sheep to fasciolisis.*

These tests carried out by V. S. Ershov on 97 lambs proved to be less successful, only 10-25 % of sheep treated with antigen from Fasciola having been found to be immune to infection. The rest experienced lowering in infectivity. Treatment of sheep with antigen gives rise only to a short term immunity to fasciolisis, the sheep infected 45 days after immunisation showing no immunity against parasites Our study of the active immunisation of domestic animals to the major helminthoses has not settled all questions as to the possibilities of applying immunisation to combat helminthoses. But there is now no

doubt that by means of antigens used for active immunisation of domestic animals it is possible to achieve, to a various extents, the immunity to major helminthoses. Active immunisation cannot. however, yet be used in protecting against animal helminthoses because of inadequate knowledge of biological properties and the chemical structure of antigens as well as due to the lack of improved methods to prepare and activate them.

It is advisable to study immunisation as a means of prophylaxis of animals only with several major helminthoses against which one can obtain the highest percentage of absolute unsensibility and a prolonged time of immunity.

Active immunisation of pigs to ascaridosis should be extensively studied as the immunisation with antigen prepared from Ascaris results in a complete immunity in 80 % of animals. Experiments had shown that the subsequent administering of intensive doses of infective eggs of Ascaris intensifies immunity and prolongs its action up to six months. Active immunisation cannot yet be used against monieziosis and fasciolosis of ruminants or ascaridiosis of hens for the following reasons:

1. The immunity in animals treated with antigens to such major helminthoses as monieziosis and fasciolosis is of short duration (only up to 1.5 months). Therefore, to effect the prophylaxis against the said parasites during a pasture season one should have had resourse to repeated vaccinations. And, last but not least, the cost of preparaing the antigen needed would be too high.

2. Only a small minority of treated animals (10 to 28 %) are safeguarded against infection as a result of active immunisation to ascaridosis and fasciolosis.

3. At the present time there have been worked out more effective and reliable procedures against major helminthoses such as chemical prophylaxis by phenothiazine against dictiocaulosis of sheep, ascaridiosis of hens and others. Encouraging results are to be expected in the use of chemical prophylaxis against fasciolosis by felixan, and so on.

RÉSUMÉ

Les auteurs ont préparé les antigènes d'ascaridia, de toxocara, d'ascarides des porcs, de distomes (Fasciola hepatica) et de moniezies (Moniezia expansa). Leur structure, chimique, avait été étudié et les auteurs, ont fait l'épreuve de leur propriétés antigenne et immunologiques sur les animaux de laboratoire et ont fait les éssais de l'immunisation active des porcelets en-

vers d'ascaridiose, des poulets envers d'ascaridiose, des agneaux envers de monieziose, des moutons envers de fasciolose.

Les antigènes présentent un complexe polysacharide et protéique, qui se compose de 60 % de la sustance de la nature polysacharide et de 30-40 % de a substance de la nature protéique.

Les antigènes ont une specificité stricte d'une éspèce et ils ont les proprietés characteristique d'antigène a valeur requise.

Après le sensibilisation des cochons d'Inde avec les antigènes correspondantes, on relève ordinairement la tipique réaction d'anaphylaxie.

Les antigènes correspondants provoquent l'élaboration des précipitines chez les animaux de laboratoire et hôtes specifiques et donnent la réaction de précipitation positive avec les homologues anticorps et anticorps du sérum sanguin des animaux infestés d'helminthes correspondants.

On peut obtenir l'immunité de different degré de la tension envers les principales helmintoses chez les animaux domestiques a l'aid des antigènes autolisés pour l'immunisation active ; on note l'insuscéptibilité complet envers d'ascaridose chez le 80 % des porcelets, envers de monieziose—chez le 60 % des agneaux, envers d'ascaridiose—chez le 28 % des poulets et envers de fasciolose chez le 10-25 % des moutons.

Cependant, à présent, on ne peut pas utiliser l'immunisation active contre les helminthiases des animaux, parce que nous ne connnaisson pas suffisanment les propriétés biologiques, la structure chimique des antigènes, parce que nous n'avons pas de méthodes complets de leur préparation et de leur activisation.

Il est utile d'étudier l'immunisation comme un méthode de prophylaxie seulement das les plus importantes helminthiases, contre lesquelles on peut obtenir le plus haut pourcentage de l'insuscéptibilité absolue et le delai plus long de l'état immune.

Il est utile de faire l'éssai de l'immunisation active envers d'ascaridose des gemacht.

ZUSAMMENFASSUNG

Es wurden von den Autoren die Antigenen von den Askaridien. Toxocarien, Schweinaskariden, Fasciolen und Moniezen vorbereitet. Sie haben auch ihre chemische Struktur untersucht, Antigens und Immunisations Eigenschaften an die Laboratoriumstiere angewendet und eine Reihe von den Experimenten der aktiven Immunisation der Ferkel zur Askaridosen, der Kükken

zur Askaridiasis, der Lammen zur Moniesiasis, der Schäfer zur Fascioliasis porcs dans les larges proportions.

Die Antigenen sind als ein Polysaccharide-Eiweisskomplex zu verstehen, der 60 % Polysaccharidestoff und 30-40 % Eiweisstoff einschliesst.

Die Antigenen besitzen ein genaues Artspezifische und typischen Eigenshaften der vollwertigen Antigenen. Bei den Meerschweinchen nach der Sensibilität mit entsprechenden Antigenen entsteht gewöhnlich eine typische anaphylaktische Reaktion.

Bei den Laboratoriumstieren und bei den spezifischen Besitzern rufen entsprechende Antigenen eine Präzipitineproduktion hervor und geben eine positive Präzipitationsreaction mit den homologischen Antikörpern und mit den Antikörpern aus dem Blutserum der Tieren, die mit entsprechenden Helminthiasis angesteckt wurden.

Mittels Antigenen, die für eine aktive Immunisation bei den landkirtschaftlichen Tieren angewendet werden, kam ein verschiedenartiger Grad des Immunitäts zu den Haupthelminthiasis erreicht werden. Eine vollständliche Unempfänglichkeit zur Askaridosis wird bei den 80 % Ferkel, zu Monesiasis bei den 60 % Lammen, zu Ascaridiasis bei den 28 % Kükken und zu Fasciliasis bei den 10-25 % Schäfer erreicht. Aber eine aktive Immunisation im Kampf gegen die Helminthiasis der Tieren kann bisher nicht verwendet, da fehlt es noch an den ausreichenden Kentnissen der biologischen Eigenschaften, chemischen Antigenstruktur und einer vollkommenen Methode der Antigenvorbereitung und ihrer Aktivierung.

Es ist zwechmässig die Immunisation, als eine Prophylaxemethode bei den Tiren, nur bei manchen Haupthelminthiasis zu erforschen. Es werden Helminthiasis gemeint, gegen den ein hohes Prozent der absoluten Unempfänglichkeit und einer dauernder Frist der Immunzustand erreicht wurde.

Es ist auch zwechmässig die Methode der aktiven Immunisation zur Ascaridiasis bei den Schweinen in den Zahlreichen Experimenten zu erforschen.

RESUMEN

Los autores han preparado antígenos de ascaridies, toxocara, ascaridies del cerdo, fasciola y moniesi, han estudiado sus estructuras químicas, han realizado experiencias de propiedades antígenas e inmunógenas sobre animales de laboratorio y experiencias de inmunización activa de lechoncillos hacia ascaridiosis, de pollos hacia acaridiosis, corderos hacia moniesiosis, ovejas hacia fasciolesis.

Antígenos son un complejo de polisacarido-albumen que incluye hasta el 60 por 100 de la sustancia polisacarida y el 30-40 por 100 de albumen.

Los antígenos tienen una especificación severa de género y tienen también propiedades de antígenos de entero valor.

Los cochinillos de Indias, después de sensibilizarlos con los antígenos correspondientes, tienen, como regla, una reacción anafiláctica.

Los antígenos correspondientes producen precipitinos de los animales de laboratorio. Ellos dan una reacción positiva de precipitación con anticuerpos homológicos y también con anticuerpos que constan del suero de sangre de los animales que tienen infección de guelmintos correspondientes.

Con ayuda de los antígenos se puede alcanzar inmunidad hacia helmintoses principales. Una inmunidad completa hacia ascaridosis tienen el 80 por 100 de los lechoncillos, el 60 por 100 de los corderos tienen inmunidad hacia moniesiosis; el 28 por 100 de los pollos, hacia ascaridiosis, y el 10-25 por 100 de las ovejas, hacia fasciolesis. Entretanto, la inmunización activa contra helmintosos de los animales no puede todavía tener un gran lugar porque no tenemos conocimientos suficientes sobre las propiedades biológicas y estructura química de los antígenos. Tampoco tenemos métodos más perfectos para la preparación y para la activización de ellos.

Estudiar inmunización como un método de la profiláctica de los animales tiene razón sólo para algunos helmintosos principales.

Racionalmente, probar el método de la inmunización activa hacia ascaridosis del puerco es una experiencia amplia.